Institute Report No. 275

Mutagenic Potential of Nitroguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

July 1988

Toxicology Series 222

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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[illegible] the research described in this report, the investigation adhered to [illegible] for the Care and Use of Laboratory Animals," as promulgated by the [illegible] of the Guide for Laboratory Animal Facilities and Care, [illegible] Laboratory Animal Resources, National Research Council.

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No. 275	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Branch Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-UL-TO-M	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 627204 / PROJECT NO. 835 / TASK NO. AB / WORK UNIT ACCESSION NO. DA 303913

11. TITLE (Include Security Classification) Mutagenic potential of nitroguanidine in the Drosophila melanogaster sex-linked recessive lethal test

12. PERSONAL AUTHOR(S) Raj K. Gupta, Lillie D. Witcher, Vilmar O.M. Villa, Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM _____ TO _____	July 1988	18

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	mutagenicity, genetic toxicology, sex-linked recess lethal assay, Drosophila melanogaster, nitroguanidine

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Nitroguanidine, a primary component of U.S. Army triple-base propellants, was evaluated for mutagenic potential in the Drosophila melanogaster Sex-Linked Recessive Lethal test. Nitroguanidine was non-mutagenic following 72-hour feeding exposures to concentrations of nitroguanidine ranging from 2.08 µg/ml to 20.8 µg/ml.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Edwin S. Beatrice, COL MC	(415) 561-3600	SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete*

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

Nitroguanidine, a primary component of U.S. Army triple-base propellants, was evaluated for mutagenic potential in the *Drosophila melanogaster* Sex-Linked Recessive Lethal test. Nitroguanidine was non-mutagenic following 72-hour feeding exposures to concentrations of nitroguanidine ranging from 2.08 µg/ml to 20.8 µg/ml.

Key Words: Mutagenicity, Genetic Toxicology, Sex-Linked Recessive Lethal Assay, *Drosophila melanogaster*, Nitroguanidine.

PREFACE

TYPE REPORT: *Drosophila melanogaster* Sex-Linked Recessive Lethal GLP Assay

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 85001

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: MAJ Raj K. Gupta, PhD, MSC

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, test compound sample, raw data, and appropriate SOPs will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 19 March 1985 - 31 December 1985

OBJECTIVE: The objective of this study was to evaluate the mutagenic potential of nitroguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Assay.

ACKNOWLEDGMENT

The investigators wish to thank Ms. Mara W. Joshua for secretarial and typing assistance.

Signatures of Principal Scientists Involved in the Study

We, the undersigned, declare that the GLP Study 85001 was performed under our supervision, according to the procedures described herein, and that the report is an accurate record of the results obtained.

Don W. Korte 29 June 88

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Study Director

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA (70-1n) 28 June 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85001

1. This is to certify that in relation to LAIR GLP Study 85001, the following inspections were made:

21 January 1985	-	Protocol Review
01 October 1985	-	F1 Cross, Brood 1, Run 55

2. The institute report entitled "Mutagenic Potential of Nitroguanidine Using the Sex-linked Recessive Lethal Test in Drosophila melanogaster," Toxicology Series 222, was audited on 24 June 1988.

Carolyn M Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of Nitroguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test--Gupta *et al.*

INTRODUCTION

Nitroguanidine, a primary component of U.S. Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The U.S. Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants generated by U.S. Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine and related intermediates/by-products of its manufacture or environmental degradation. This study evaluated the mutagenic potential of nitroguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal assay.

Rationale for SLRL Testing

A variety of tests using *Drosophila melanogaster* are available for the detection of specific types of genetic changes. The most sensitive assay that detects the broadest range of mutations is the SLRL test (2-4). This test uses insects of a known genotype and detects lethal mutagenic changes in 800 to 1000 loci on the X-chromosome, representing 80% of the X-chromosome or 20% of the entire genome (5,6). The SLRL test is used frequently to assess the mutagenic response of *Drosophila melanogaster* to test substances (2,4,5).

Genetic Basis of the SLRL Test

The basic mechanism of the SLRL test is that the X-chromosome of the father is passed on to the daughter; the sons receive their X-chromosome from the mother. Therefore, the recessive lethal mutations located on the X-chromosomes are expressed in males in a hemizygous condition, i.e. the Y-chromosome does not contain the dominant, wild-type alleles to suppress the manifestation of recessive lethal mutations.

Consequently, among the progeny of a female carrying a recessive lethal mutation on one of her X-chromosomes (heterozygous for a recessive lethal mutation), half of the male progeny die. By using suitable genetic markers, the class of males carrying the mutated X-chromosomes of treated grandfathers can be determined easily. If a lethal mutation were induced, this class would be missing and its absence easily scored. The SLRL test has also been called the Basc or Muller-5 test (6,7). The assay system must use strains in which crossing-over in females is prevented since transfer of the lethal mutation from the paternal to the maternal X-chromosome by genetic recombination would suppress its expression. The crossing-over would lead to erroneous study results because males receiving that X-chromosome would survive. Since combinations of suitable inversions effectively inhibit crossing-over, females used for the SLRL test carry two scute inversions: the left-hand part of sc^{S1} and the right-hand part of sc^{8} covering the whole X-chromosome and a smaller inversion In-S in the Basc chromosome (6).

Description of Test

The SLRL test (8) was developed in 1948 for determining genetic changes that kill the developing individual (egg to pre-adult stage) in the hemizygous, but not homozygous or heterozygous, conditions. Such genetic changes, i.e. recessive lethal mutations, can be induced on all chromosomes. Only two test generations are needed to detect whether sex-linked recessive lethal mutations have been induced on the X-chromosome. In the test, males with normal round red eyes (Canton-S (CS)) whose chromosomes contain wild-type alleles are exposed to nitroguanidine. Such an exposure will produce a recessive lethal mutation if the X-chromosome is affected. These males are mated to homozygous Basc females. The Basc phenotype is characterized by bar (narrow-shaped) eyes which are apricot in color. The bar eyes serve as a genetic marker for the homozygous and hemizygous genotypes since in the heterozygous expression the eyes are kidney-shaped. The progeny of this cross (CS males X Basc females) consists of females heterozygous for the treated X-chromosome, characterized by kidney-shaped red eyes and males of the Basc phenotype that have received their X-

chromosome from their Basc mother. Each F1 female possesses one paternal X-chromosome which was exposed to the test compound in the male gamete. F1 siblings are allowed to mate; they produce the F2 generation. The F2 generation will consist of males of two phenotypic expressions and females of two phenotypic expressions. The male phenotypes have round red eyes (hemizygous carrying the treated X-chromosome from the F1 female) or bar-shaped apricot eyes (hemizygous for the Basc chromosome). The female phenotypes carry the chromosomes for red eyes (heterozygous, carrying the treated X-chromosomes from the F1 female and the Basc chromosome) or chromosomes for bar-shaped apricot eyes (homozygous for the Basc chromosome). The F2 generation is then inspected for the presence of males with round eyes. If this class is missing, it can be concluded that the treated male gamete contained a recessive lethal mutation. Thus, this test relies upon the disappearance of a whole Mendelian class (males with round red eyes).

A brooding technique was used to sample sperm cells exposed to the test chemical during different stages of spermatogenesis because chemicals often exhibit stage specific mutagenicity. Brooding was accomplished by transferring the treated males to vials containing fresh virgin females at intervals of 1, 4, 6, and 8 days after completion of the dosing period. This technique assures that the four broods of females are inseminated with sperm exposed to the test chemical during successive stages of germ cell development: Brood 1 = mature sperm (Days 1-3); Brood 2 = primarily spermatids (Days 4-5); Brood 3 = primarily meiotic stages (Days 6-7); and Brood 4 = primarily spermatogonia (Days 8-10). This procedure safeguards against the possibility that chemicals with more pronounced effects in earlier stages of spermatogenesis are not dismissed erroneously as false negatives.

Objective of Study

The objective of this study was to evaluate the mutagenic potential of nitroguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Assay.

MATERIALS AND METHODS

Test Substance

Chemical Name: Nitroguanidine

CAS Registry #: 556-88-7

Molecular formula: $CH_4N_4O_2$

Other test substance information is presented in Appendix A.

Vehicle

A number of compounds were tested as a vehicle for their solubility, stability, and toxicity. A solution of 1% fructose in water was found to be an appropriate vehicle for the nitroguanidine.

Test Model

Insect Genus and Species: *Drosophila melanogaster*

Strains: Canton-S (CS), a wild-type stock, characterized by round red eyes, was selected for mutagenicity studies because it has shown a low spontaneous mutation frequency (9).

Basc, a laboratory stock, homozygous in females, possesses bar-shaped, apricot-colored eyes and scute as phenotypic markers. The genetic designation is $In(1)sc^{S1}L sc^{8R} IN(1)S$, sc^8, $sc^{S1} w^a B$.

Both strains are presently being reared in the insectary at Letterman Army Institute of Research. The original stock colonies were obtained from the Mid-American *Drosophila* Stock Center, Bowling Green State University, Bowling Green, Ohio.

Diet

The diet was the standard medium consisting of cornmeal (NBCO Chemicals), unsulphured molasses (Ingredient Technology Corp.), Yeast (Nabisco Brands, Inc.), and nutrient agar (Moorhead & Co., Inc.) used for colony rearing of *Drosophila melanogaster*. A materials list and instructions for its preparation are contained in LAIR SOP-OP-STX-5 "Drosophila Media Preparation."

Restraint

Ether (J. T. Baker Chemical Co.) anesthesia was used to restrain flies being collected for mating and for general colony maintenance.

Identification System

Each CS male from the 72-hour LC_{50} exposure (test, negative, and positive control) was assigned a unique number. This number was also placed on the vial in which its progeny was produced (LAIR SOP-OP-STX-8 "Sex-Linked Recessive Lethal (SLRL) *Drosophila melanogaster* Mutagenicity Test"). In this manner progeny could be traced back to the parental male which had been subjected to the test compound or control vehicle.

Storage of Raw Data

Tabular data from this study (GLP 85001) for each male are in the archives of Letterman Army Institute of Research, Presidio of San Francisco, California.

Environmental Conditions

All studies were conducted within the insectary at a temperature of 23 ± 2°C, relative humidity of 57 ± 8% and a photoperiod of 12 hours light and 12 hours dark. All insect colonies were reared in polypropylene bottles, and those used in the SLRL testing were housed in glass vials (LAIR SOP-OP-STX-6 "Drosophila Stock Colony Maintenance").

Dosing

The solubility of nitroguanidine in water is relatively high compared to that of most explosives (10). Preliminary studies were conducted to test potential dosages for toxicity to flies and their ability to feed and digest nitroguanidine in an appropriate medium. A feeding solution vehicle of 1.0% fructose in deionized water was selected for the study.

Dosing was accomplished in compliance with LAIR SOP-OP-STX-7 "*Drosophila melanogaster* Exposure Procedures." The CS strain (wild-type) males were allowed to feed on 250 µl of various concentrations of the test chemical. These males formed the test groups. Concurrent exposure to 1.0% fructose in deionized water was designated as the negative control group. A positive control group was exposed to a 1.0-mM ethylmethane sulfonate solution formulated with 1.0% fructose in water. Ethylmethane sulfonate is a known mutagen and was used to confirm the ability of the test organism to produce SLRL mutations (11). Dosing was continuous for 72 hours. Flies were transferred every 24 hours to vials containing

fresh compound solutions. The concentration of nitroguanidine tested for toxicity ranged from 2.08 µg/ml to 20.8 µg/ml.

Test Format

The CS males surviving the LC_{50} (approximate) dose of the test chemical and those males subjected to the concurrent negative controls were used in the SLRL assay. Twenty-five CS male (wild-type) survivors of the test chemical and negative control compound were scored by mating to Basc virgin females (Basc chromosomes). This procedure was accomplished by placing 3 Basc virgin females in a vial with one CS male. The vial was labeled with the male's unique number. At days 1, 4, 6, and 8 after dosing the CS male was transferred to successive groups of 3 Basc virgin females in vials with that male's unique number. These intervals corresponded to broods 1, 2, 3, and 4. This procedure was replicated 4 times. Scoring of the mutants resulting from positive control exposure was based on mating 5 CS males in the same manner as males treated with the test compound. This procedure was also replicated 4 times. After sufficient numbers of flies had emerged, a maximum of 25 (minimum of 5) kidney-shaped red-eyed F1 females were selected at random and mated with their sibling white-body, bar-shaped, apricot-eyed males. Each pair was placed in an individual vial, and these vials from the same uniquely numbered father were placed together and labeled with his unique number for reference. After 2 to 3 weeks the F2 progeny were examined and scored for the absence of round, red-eyed males, which would indicate that a lethal mutation had taken place in the treated male. Confirmation of a lethal mutation was obtained by conducting an F3 cross from each vial scored as a lethal mutation. This was accomplished by crossing three F2 females (kidney-shaped red eyes) with three males with bar-shaped white-apricot eyes, in three separate vials (one male and one female per vial). Absence of males with round, red eyes in the resulting F3 generation confirmed the existence of a recessive lethal mutation. Experimental conclusions were based on the spontaneous mutation frequency (negative control) compared to the mutation frequency induced by the test chemical. This entire procedure was replicated 4 times to obtain a sufficiently large sample.

Historical Listing of Significant Study Events

Appendix B is a historical listing of significant study events.

Statistical Analysis

This testing was designed to examine approximately 2500 X-chromosomes in each of 4 replications, thereby yielding a

This testing was designed to examine approximately 2500 X-chromosomes in each of 4 replications, thereby yielding a total of 8000 to 10,000 X-chromosomes for examination. Vials without F2 progeny or fewer than 5 progeny (F2) were scored as failures. The BMDP (Biomedical Programs) computer package was used to perform the analyses (12). Based on the number of lethal and nonlethal offspring for each male, by combining all replicates, the mutation frequency of nitroguanidine was compared to that of the control by Fisher's exact test for each of the four broods separately and for the combined broods. All statistical tests were conducted at the 0.05 level of significance.

Deviations from SOP/Protocol

The following deviations from the Standard Operating Procedures and GLP Protocol 85001 were made during this study:

The CS males were dosed at a concentration determined in the preliminary studies. The maximum soluble amount that provided a homogeneous solution was administered because of the low toxicity of nitroguanidine.

The total number of flies examined in the test group was 6913. For the negative control group, the number of flies examined was 7318. Although less than 8000, both sample sizes were considered adequate for the individual brood test using Fisher's exact test.

Use of dimethyl sulfoxide (DMSO) as a solvent was avoided as recommended by the EPA (13). These deviations from the Standard Operating Procedures had no effect on the outcome of the study.

RESULTS

The frequencies of spontaneous mutation for nitroguanidine and the negative control were 0.188% and 0.096% based on 6913 and 7318 X-chromosomes, respectively. The mutation frequencies for the positive control, 1-mM ethylmethane sulfonate, was 17.8%. The mutation frequencies for each compound are presented in Table 1. The mutation frequencies for each brood for nitroguanidine and the negative control are presented in Table 2. No significant difference was detected between the mutation frequency of the negative control and the nitroguanidine with the Fisher's exact test ($p = 0.1799$). Also, no significant differences

Table 1

PERCENT MUTATION FREQUENCIES IN THE SEX-LINKED RECESSIVE LETHAL ASSAY OF NITROGUANIDINE*

Compound	1	2	3	4	Total Mutations	(Percent) Mutations
Nitroguanidine	0/1115	5/1231	4/2352	4/2215	13/6913	(0.188)
Negative Control	1/1481	1/1529	3/2155	2/2153	7/7318	(0.096)
Positive Control	36/166	39/284	56/395	70/284	201/1129	(17.80)

*Data are recorded as number of SLRL events/number of X-chromosomes tested.

Nitroguanidine: 25 male *Drosophila melanogaster* flies (CS strain) formed the P generation.

Negative Control: 25 male *Drosophila melanogaster* flies (CS Strain) formed the P generation.

Positive Control: 5 male *Drosophila melanogaster* flies formed the P generation.

Table 2

FISHER'S EXACT TEST FOR SIGNIFICANCE OF THE DIFFERENCE BETWEEN NITROGUANIDINE AND NEGATIVE CONTROL IN SEX-LINKED RECESSIVE LETHAL ASSAY

	Brood Number			
Compound	1	2	3	4
Nitroguanidine	3/2055	3/1815	5/1727	2/1316
Negative Control	2/2094	2/1901	1/1844	2/1479
Positive Controls	44/336	88/291	63/316	6/186
p values	0.6845	0.6805	0.1138	1.0000

Nitroguanidine: Nitroguanidine was dissolved in a 1% fructose solution in deionized H_2O. Data are from 25 male *Drosophila melanogaster flies (CS strain)* x 4 replicates mated with 3 Basc strain female flies each.

Negative Control: 1% fructose in deionized water. Data are from 25 *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

Positive Control: 1.0 mM ethylmethane sulfonate and 1% fructose in deionized H_2O. Data are from 5 male *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

were detected between the negative control and the nitroguanidine for the data of broods 1, 2, 3, and 4 (Table 2).

DISCUSSION

Nitroguanidine has been reported to produce significant chromosome damage in Chinese hamster fibroblasts (14). However, studies from this laboratory have indicated that nitroguanidine is not mutagenic in the mouse lymphoma forward mutation assay, the Chinese hamster ovary sister chromatid exchange assay, or in the Ames *Salmonella* mammalian microsome assay (15). The results of this study confirm in an *in vivo* model for genetic toxicity our previous findings that nitroguanidine has no mutagenic potential at doses which approach the limits of solubility in the appropriate test systems.

CONCLUSION

The results of this study indicate that nitroguanidine is not mutagenic when evaluated in the *Drosophila melanogaster* sex-linked recessive lethal assay.

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APPENDICES

Appendix A: CHEMICAL DATA

Chemical Name: Nitroguanidine (NGu)

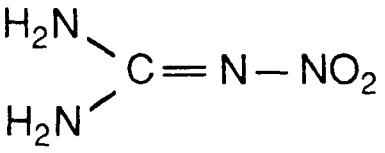
Other Listed Names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

Lot Number: SOW83H001-004

LAIR Code: TP36

Chemical Structure:



Molecular Formula: $CH_4N_4O_2$

Molecular Weight: 104.1

Physical State: White powder

Melting Point: 232° C[1]

Names of Contaminants and Percentages: (Data Sheet Attached)

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Analytical Data:
An infrared spectrum was obtained upon receipt of the compound; major absorption peaks were observed at 3330 (broad), 1660, 1630, 1525, 1400, 1300, 1050, and 780 cm^{-1}.[2] The spectrum was identical to the Sadtler spectrum for nitroguanidine.[3]

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[1]Fedoroff BT, Sheffield OE. Encyclopedia of explosives and related items. Vol 6. Dover, New Jersey: Picatinny Arsenal, 1975: G154.

[2]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 39. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1967: Infrared spectrogram #21421.

Appendix A (Contd.): CHEMICAL DATA

Stability:

An aqueous solution of NGu (48.1 μmolar) was prepared and the absorption at 264 nm determined to be 0.689 AUFS. Three weeks later the same solution was reexamined spectroscopically and the absorption at 264 nm found to be 0.689 AUFS. A full spectrum scan revealed the characteristic pattern of absorption in the UV range with peak maxima at 215 and 264 nm. These data indicate that NGu is stable in aqueous solution for at least three weeks.[4]

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[4]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010, pp 22 and 24. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (Contd.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC
(DRSAR-PPI-69)

CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE: 1 OF 1

TO: Commander, US Army Armament Munitions and Chemical Command, Attn: DRSMC-PAD, Rock Island, Ill. 61201

FROM: Sunflower Army Ammunition Plant, DeSoto, Kansas 66018

DATE: September 13, 1983

MATERIAL: Nitroguanidine, Type II, Class 2 *

MANUFACTURER: Hercules Aerospace Division, Hercules Incorporated

CONTRACT NO.: DAAA-09-77-C-4016, CLIN 0010

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
SOW83H001-004		1	7,000 lbs.

PLACE MANUFACTURED: Sunflower Army Ammunition Plant, DP Facility

SPECIFICATION AND AMENDMENT/DRAWING NO.: MIL-N-494A w/Int. Amend 6 (AR) Dated 25 March 1981 *

SECTION B - DESCRIPTION OF MATERIAL

Property	Requirement Min.	Max.	Analysis
Purity, %	99.0		99.6
Ash Content, %		0.30	0.03
pH Value	4.5	7.0	7.55 **
Acidity (as H_2SO_4), %		0.06	ND ***
Total Volatiles, %		0.25	0.03
Sulfates (as $NaSO_4$), %		0.20	0.01
Impurities, H_2O Insoluble, %		0.20	0.01
Particle Size, Microns		3.0 *	4.0 ****
Particle Size, Std. Dev.		± 0.5	0.168

* As amended by Contract Scope of Work
** Approved by Waiver No. NQ83-1 dated Sept. 2, 1983
*** ND = None Detected
**** Approved by Waiver No. NQ83-2 dated Sept. 9, 1983

REMARKS

1.) Manufactured under SOW ES 1A-3-8423, Nitroguanidine Particle Size, dated 1 Feb. 83.

2.) Packaging: Level B - fiber drums to Spec. DOT 21C60. Drums numbered 3 thru 243 and 247 thru 285. 25 pounds per drum per HAD letter dated Aguust 1, 1983, to COR.

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: Hercules Aerospace Division

TESTING CONDUCTED BY: Hercules Aerospace Division

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

13 Sept 83 — DATE — M. A. KORAD, CERTIFYING SIGNATURE

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

14 Sept 83 — DATE — Quality Assurance Specialist — TITLE

FOR THE COMMANDER

M. A. Korad — SIGNATURE

ARRCOM FORM 213-R, 10 AUG 77 — REPLACES ARMCOM FORM 213-R, 15 JUL 71, WHICH MAY BE USED.

Appendix B. HISTORICAL LISTING OF SIGNIFICANT EVENTS

Date	Event
29 April - 3 May 85	Begin Replicate 1 (Run 58).
24 June - 28 June 85	Begin Replicate 2 (Run 59).
9 Sept - 13 Sept 85	Begin Replicate 3 (Run 60).
18 Nov - 22 Nov 85	Begin Replicate 4 (Run 61).

Distribution List

Commander
US Army Biomedical Research and Development Laboratory **(27)**
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) **(2)**
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command **(2)**
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

6/88

END

DATE

FILMED

DTIC

10-88